A CONTRIBUTION TO THE PROPHYLAXIS OF LOBAR PNEUMONIA

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While the earliest investigations in the bacteriology of pneumonia have shown the presence of pneumococci in the mouth secretions of healthy persons as well as those suffering with lobar pneumonia, recent investigations show that the disease is frequently acquired by transmission of specific virulent types of pneumococci from a recovered person who still harbors in the mouth-secretions the organisms responsible for the disease, or from a healthy carrier of pathogenic pneumococci. Dochez and Avery[1] found about 40% of healthy persons intimately associated with cases of lobar pneumonia harboring the same types of pneumococci as those producing the disease; these carriers included physicians, nurses, and members of the patients' families as wives, mothers, brothers and sisters. Convalescents from pneumonia were found to carry the type of pneumococcus with which they have been infected, for a considerable length of time — in one instance as long as 90 days, counting from the first day of illness.

Stillman[2] found pneumococci in the mouths of 172 normal persons of 398 studied; in 8 of these persons Types I and II pneumococci were present, while 26 persons harbored typical Type II organisms and 44 Type III. Of 84 persons studied who had been in contact with cases of pneumonia, 5, or 5.95%, showed either pneumococci of Type I or Type II in their sputum; the shortest period of carrying was 7 days, the longest 85 days. Sydenstricker and Sutton[3] found from 6-22% of healthy persons harboring pneumococci of the fixed types in the sputum and about 32% harboring the ordinary saprophytic Type IV organisms. In a more recent summary of studies of a large number of healthy individuals in contact with cases of lobar pneumonia due to Type I and

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[1] Jour. Exper. Med., 1915, 22, p. 105.

[2] Ibid., 1916, 24, p. 651.

[3] Bull. Johns Hopkins Hospital, 1917, 28, p. 312.

Type II pneumonococci, Avery, Chickering, Cole and Dochez[4] report about 12% were found to carry the corresponding types of pneumococci, whereas among healthy persons not in contact with cases of lobar pneumonia, these types were found in but 0.3 or less per cent.

These investigations having definitely established the existence of the carrier state of disease producing pneumococci among healthy persons and those recently recovered from pneumonia, and indicating the means by which lobar pneumonia may be spread, it would appear advisable to attempt the destruction of pneumococci in the mouth secretions particularly among convalescents and those in intimate contact with cases of lobar pneumonia. We have approached this problem mainly from the laboratory side bearing in mind the numerous failures which have followed clinical attempts to disinfect mucous membranes and secretions of various bacteria.

As the investigations of Morgenroth and Levy,[5] Wright,[6] Moore,[7] Cohen, Kolmer and Heist[8] have shown the high pneumococcidal activity of cinchona derivatives and particularly ethylhydrocuprein, we have made our studies on the disinfection of sputum and the mouth with solutions of various cinchonics in a menstruum of liquor thymolis * our experiments demonstrating that the latter solution alone possesses some germicidal activity for pneumococci and aids in disguising the bitter taste of cinchona compounds. Wadsworth[9] has tested the power of saline solutions and bland alcoholic washes in ridding the mouth of pneumococci, but no specific pneumococcidal substances were used; his results, however, were rather encouraging.

In our experiments both normal mouth secretions harboring Type IV pneumococci and the sputum of pneumonia convalescents harboring Type I organisms were employed and the pneumococcidal activity of the substance under experiment determined largely by mouse inoculation.

Preliminary mouse inoculation tests with the mouth secretions of several normal persons who had not recently been in contact with cases of pneumonia showed that the intraperitoneal injection of 1 c.c. of their sputum produced

[4] Monographs of the Rockefeller Institute, 1917, No. 7, Acute Lobar Pneumonia.
[5] Berl. klin. Wchnschr., 1911, 48, pp. 1561, 1650, 1779, 1983.
[6] Lancet, 1912, 11, pp. 1633, 1701.
[7] Jour. Exper. Med., 1915, 22, p. 269.
[8] Jour. Infect. Dis., 1917, 20, p. 272.

* Liquor thymolis used in the Polyclinic hospital as a substitute for liquor antisepticus, is prepared as follows: benzoic acid, 64 grains; boric acid 128 grains; thymol and menthol, each 16 grains; oil of eucalyptus, oil of wintergreen and oil monarda each 4 drops; alcohol and glycerin, each 4 ounces; water sufficient for 16 ounces.

[9] Ibid., 1906, 3, p. 774.

death in about 24 hours with Type IV pneumococci in the peritoneal cavity and blood of the heart; the injection of 0.25-0.5 c.c. of sputum killed at irregular intervals but usually within 4 days.

Experiments conducted with a centrifuge method previously described by Kolmer[10] yielded results as shown in Table 1. In conducting these tests the mouth secretions of one or more normal persons were collected in sterile vials and 1 c.c. placed in a series of sterile centrifuge tubes with 1 c.c. of the varying dilutions of a cinchonic made up with liquor thymolis; after thorough mixing, the tubes were incubated at 35 C. for 10 minutes, thoroughly centrifuged and the supernatant fluids discarded. To the sediment in each tube 1.5 c.c. of sterile salt solution was added and 1 c.c. injected intraperitoneally in mice and 0.2 c.c. plated with blood agar.

TABLE 1

THE GERMICIDAL ACTIVITY OF VARYING DILUTIONS OF CINCHONICS IN 1:4 LIQUOR THYMOLIS ON TYPE IV PNEUMOCOCCI IN MOUTH SECRETIONS OF NORMAL PERSONS (CENTRIFUGE TESTS)

Cinchonic	Final Dilutions of Cinchonic	Animal Tests			Plates 48 Hour Counts
		24 Hour	48 Hour	72 Hour	
Ethylhydrocuprein	1:10,000	S*	S	S	5,700
	1:16,000	S	S	S	7,900
	1:20,000	S	S	S	Uncountable
	1:32,000	D			Uncountable
Quinine bisulphate	1:10,000	S	S	D	Uncountable
	1:16,000	S	D		Uncountable
	1:20,000	D			Uncountable
Quinine hydrobromid	1:10,000	S	S	D	4,000
	1:16,000	D			Uncountable
	1:20,000	D			Uncountable
Liquor thymolis	1:45	S	D		Uncountable
Sputum alone		D†			Uncountable

* S = survived; D = died.
† Type IV pneumococci in peritoneal exudate.

As shown in Table 1, dilutions of ethylhydrocuprein hydrochlorid as high as 1:20,000 had well marked germicidal activity for Type IV pneumococci in the mouth secretions of normal persons; quinin bisulphate and quinin hydrobromid exhibited less activity, but the effects were usually distinct with dilutions reaching 1:10,000. Not infrequently liquor thymolis alone in a 1:4 dilution with the sputum showed feeble pneumococcidal activity; plates showed some germicidal influence on the ordinary bacterial flora of mouth secretions and particularly on pneumococci on the part of the lower dilutions of the various compounds, but these results are to be ascribed mostly to the 1:4 dilution of liquor thymolis alone and particularly in so far as these bacteria other than the pneumococci are concerned.

[10] Ibid., 1917, 20, p. 294.

The results of additional experiments conducted after the in vitro-vivo method previously described[10] are shown in Tables 2 and 3.

In these tests thorough mixtures of the mouth secretions of normal persons or the sputum of pneumonia convalescents were made with varying dilutions of cinchonics in a menstruum of liquor thymolis and incubated for varying intervals of time when the pneumococcidal activity was studied by injecting portions intraperitoneally into mice and by plating methods. This technic had

TABLE 2

GERMICIDAL ACTIVITY OF VARYING DILUTIONS OF ETHYLHYDROCUPREIN HYDROCHLORID IN 1:4 LIQUOR THYMOLIS UPON PNEUMOCOCCIC TYPE IV IN THE SPUTUM OF NORMAL PERSONS *

Sputum C.c.	Cinchonic in 1:2 Liq. Thymolis	Final Dilutions in 1:4 Liq. Thymolis	Animal Tests			48 Hour Blood Agar Plates
			24 Hour	48 Hour	72 Hour	
0.5	0.5 c.c. 1: 2,000	1: 4,000	D†			Sterile
0.5	0.5 c.c. 1: 5,000	1:10,000	S‡	S	S	Sterile
0.5	0.5 c.c. 1: 8,000	1:16,000	S	S	S	Sterile
0.5	0.5 c.c. 1:10,000	1:20,000	S	S	S	Sterile
0.5	0.5 c.c. liq. thymolis alone	1:4 liq. thymolis alone	S	S	S	Few colonies
0.5	0.5 c.c. salt solution	Sputum control	S	S	D§	Uncountable

* Mixtures incubated in thermostat at 35 C. for 10 minutes; 0.5 c.c. injected intraperitoneally and 0.2 c.c. in each plate.
† D = died with convulsions due to toxicity of compound.
‡ S = survived.
§ Control died in 80 hours; Type IV pneumococci in peritoneum and heart.

TABLE 3

GERMICIDAL ACTIVITY OF VARYING DILUTIONS OF QUININ HYDROBROMID IN 1:4 LIQUOR THYMOLIS UPON PNEUMOCOCCI TYPE IV IN THE SPUTUM OF NORMAL PERSONS *

Sputum C.c.	Cinchonic in 1:2 Liq. Thymolis	Final Dilutions in 1:4 Liq. Thymolis	Animal Tests			48 Hour Blood Agar Plates
			24 Hour	48 Hour	72 Hour	
0.5	0.5 c.c. 1: 2,000	1: 4,000	D†			Sterile
0.5	0.5 c.c. 1: 5,000	1:10,000	S‡	S	S	Sterile
0.5	0.5 c.c. 1: 8,000	1:16,000	S	S	S	Sterile
0.5	0.5 c.c. 1:10,000	1:20,000	S	S	S	Few colonies
0.5	0.5 c.c. liq. thymolis alone	1:4 liq. thymolis alone	S	S	S	Few colonies
0.5	0.5 c.c. salt solution	Sputum control	S	S	D§	Uncountable

* Mixtures incubated in thermostat at 35 C. for 10 minutes; 0.5 c.c. injected intraperitoneally and 0.2 c.c. in each plate.
† D = died with convulsions due to toxicity of compound.
‡ S = survived.
§ Control died in 80 hours; Type IV pneumococci in peritoneum and heart.

previously yielded us constant and well defined results in a study of the germicidal activity of various cinchonics for pure cultures of various types of pneumococci and in these experiments the results were also of a clear cut character.

Mouth secretions or sputum in amounts of 0.5-1.0 c.c. were mixed in sterile vials with an equal amount of varying dilutions of cinchonics dissolved in diluted liquor thymolis and incubated at 35-37 C. for 10-30 minutes when 0.5 c.c. of each mixture was injected intraperitoneally into white mice and 0.2 c.c. plated; the usual controls of sputum alone and of sputum with liquor thymolis alone were always included.

As shown in Tables 2 and 3, the liquor thymolis alone in dilution of 1:4 or 25% protected the mice against the relatively small dose of sputum (0.25 c.c.) of normal persons containing Type IV pneumococci and in conjunction with the two cinchonics employed, namely, ethylhydrocuprein hydrochlorid and quinin hydrobromid, exerted a well marked bactericidal effect as determined by plating the mixtures. The deaths of the mice receiving injections of the 1:4,000 dilutions of both compounds were ascribed to the combined toxicity of the drugs and liquor thymolis; the blood of the heart in all such instances was sterile.

The results of a second series of experiments conducted with pneumonic sputum containing virulent Type I pneumococci are shown in Tables 4 and 5. As a 1:4, or 25%, solution of liquor thymolis is rather too strong for the purpose of gargling or washing the mouth, these tests were conducted with a 1:10 dilution of liquor thymolis in water, the final dilutions after mixing with equal parts of sputum being 1:20, or 5%. In these experiments the mixtures of sputum and varying dilutions of cinchonics were incubated in a water bath at 37 C. for one-half hour when 0.5 c.c. of each of the mixtures and controls (equal to 0.25 c.c. undiluted sputum) were injected intraperitoneally into mice and 0.2 c.c. of each plated with blood dextrose agar.

As shown by the results of these experiments the solutions of ethylhydrocuprein hydrochlorid and quinin bisulphate had an appreciable effect in prolonging the lives of many of the mice. In all instances the sputum controls died within 24 hours with Type I pneumococci in the peritoneal cavity and blood of the heart; likewise, the 1:20, or 5%, solution of liquor thymolis alone had no appreciable influence in protecting mice against these virulent pneumococci. While a number of the mice injected with the mixtures of cinchonics and sputum died at irregular intervals as so commonly occurs in tests of this kind, the results of numerous experiments indicated that dilutions of ethylhydrocuprein hydrochlorid in sputum as high as 1:30,000 and even to 1:160,000 had appreciable and frequently well defined pneumococcidal activity, while a 1:10,000 dilution almost invariably protected the mice indefinitely. With such cinchonics as quinin bisulphate, dilutions in sputum varying from 1:10,000 to 1:20,000 were found to possess well defined pneumococcidal activity.

Regarding the bactericidal activity of these dilutions of ethylhydrocuprein hydrochlorid and other cinchonics in 1:20 liquor thymolis on the other types of bacteria found in the mouth secretions of normal

and pneumonic persons, our experiments with plating methods were generally negative; as stated and shown in Tables 1, 2, and 3, stronger dilutions of liquor thymolis have an appreciable germicidal activity on these mouth bacteria. Additional experiments have shown that

TABLE 4

GERMICIDAL ACTIVITY OF VARYING DILUTIONS OF ETHYLHYDROCUPREIN HYDROCHLORID IN 1:20 LIQUOR THYMOLIS ON PNEUMONIC TYPE I SPUTUM *

Sputum C.c.	Cinchonic in 1:10 Liq. Thymolis	Final Dilutions in 1:20 Liq. Thymolis	Animal Tests: Days						Plates
			1	2	3	4	5	6	
0.5	0.5 c.c. 1: 5,000	1: 10,000	S	S	S	S	S	S	Uncountable
0.5	0.5 c.c. 1: 8,000	1: 16,000	S	S	D‡				Uncountable
0.5	0.5 c.c. 1:10,000	1: 20,000	D‡						Uncountable
0.5	0.5 c.c. 1:16,000	1: 32,000	S	S	S	S	S	S	Uncountable
0.5	0.5 c.c. 1:40,000	1: 80,000	S	D‡					Uncountable
0.5	0.5 c.c. 1:80,000	1:160,000	S	S	S	D‡			Uncountable
0.5	0.5 c.c. liquor thymolis 1:10	1:20 liq. thymolis alone	D						Uncountable
0.5	0.5 c.c. salt solution	Sputum control	D†						Uncountable

* Mixtures incubated in water bath at 37 C. for 30 minutes.
† Control; Type I pneumococci in peritoneal cavity and blood of heart.
‡ Type I pneumococci in blood of heart.

TABLE 5

GERMICIDAL ACTIVITY OF VARYING DILUTIONS OF QUININ BISULPHATE IN 1:20 LIQUOR THYMOLIS ON PNEUMONIC TYPE I SPUTUM *

Sputum C.c.	Cinchonic in 1:10 Liq. Thymolis	Final Dilutions in 1:20 Liq. Thymolis	Animal Tests: Days						Plates
			1	2	3	4	5	6	
0.5	0.5 c.c. 1: 5,000	1: 10,000	S	S	D‡				Uncountable
0.5	0.5 c.c. 1: 8,000	1: 16,000	S	S	S	D‡			Uncountable
0.5	0.5 c.c. 1:10,000	1: 20,000	S	S	S	S	S	S	Uncountable
0.5	0.5 c.c. 1:16,000	1: 32,000	D‡						Uncountable
0.5	0.5 c.c. 1:40,000	1: 80,000	D‡						Uncountable
0.5	0.5 c.c. 1:80,000	1:160,000	D‡						Uncountable
0.5	0.5 c.c. liquor thymolis 1:10	1:20 liq. thymolis alone	D‡						Uncountable
0.5	0.5 c.c. salt solution	Sputum control	D†						Uncountable

* Mixtures incubated in water bath at 37 C. for 30 minutes; 0.5 c.c. of each injected intraperitoneally into mice.
† Control; Type I pneumococci in peritoneal cavity and heart blood.
‡ Type I pneumococci in heart blood.

dilutions of liquor thymolis with sputum equal to 1:2 and 1:4 are strongly germicidal for pneumococci and other micro-organisms after an exposure of 10 minutes at 37 C., but dilutions of 1:8 and 1:10 and higher under the same conditions possess feeble or no appreciable germicidal activity.

After many trials we found that 1:10,000 dilutions of ethylhydrocuprein hydrochlorid or of quinin bisulphate, quinin hydrobromid and other cinchonics in a 1:10 dilution of liquor thymolis, constitute mixtures which could be readily used by persons as a mouth wash and gargle. The slightly bitter taste remaining after the use of any of these washes is readily removed by rinsing the mouth with plain water. Further experiments were conducted with two such washes prepared as follows:

Quinin bisulphate	0.005	gm.
Liquor thymolis	5.0	c.c.
Aqua destillata.....q. s.	50.0	c.c.

Ethylhydrocuprein hydrochlorid...	0.005	gm.
Liquor thymolis	5.0	c.c.
Aqua destillata.....q. s.	50.0	c.c.

Mixtures of equal parts of these 1:10,000 solutions of quinin bisulphate and ethylhydrocuprein hydrochlorid in 1:10 liquor thymolis were made with the sputums of persons convalescent from lobar pneumonia containing virulent Type I pneumococci and also with the mouth secretions of normal persons harboring Type IV pneumococci and incubated in a water bath at 37 C., when 0.5 c.c. of each were injected intraperitoneally in mice at intervals of 1, 2, 3, 5, and 10 minutes to determine the effect on the pneumococci present according to the duration of the lives of the experimental animals. As each mouth wash was diluted with an equal part of sputum in these experiments, the results indicate the influence of 1:20,000 dilutions of quinin bisulphate and ethylhydrocuprein hydrochlorid, respectively, in 1:20 liquor thymolis, on the pneumococci in both types of sputum.

The results of such experiments with pneumonic convalescent sputum are given in Tables 6 and 7.

With virulent Type I sputums of pneumonia convalescents the mouth wash of quinin bisulphate had but slight effect, prolonging the lives of many mice for but 24-30 hours beyond the duration of life among the controls receiving equal doses of sputum alone (Table 6); the results with ethylhydrocuprein hydrochlorid were usually more marked and particularly with the longer periods of exposure (Table 7).

Similar experiments with the mouth secretions of normal persons harboring virulent Type I pneumococci have not been made, but we

believe that the results would be quite similar to those shown in Tables 6 and 7.

While these experiments have demonstrated the pneumococcidal activity of 1:10,000 solutions of ethylhydrocuprein hydrochlorid and quinin bisulphate in 1:10 liquor thymolis, a few experiments on the disinfection of the mouths of normal persons harboring Type IV pneumococci have been generally negative. Washing the mouth for

TABLE 6

GERMICIDAL ACTIVITY OF MOUTH WASH OF A 1:20,000 DILUTION OF QUININ BISULPHATE IN 1:20 LIQUOR THYMOLIS ON PNEUMONIC SPUTUM CONTAINING VIRULENT TYPE I ORGANISMS

Exposure at 37 C.	Animal Tests; Days			
	1	2	3	4
1 minute	D*			
2 minutes	S	D*		
3 minutes	S	D*		
5 minutes	D			
10 minutes	S	D*		
Control (1 minute)	D*			
Control (10 minutes)	D*			

* Type I pneumococci in peritoneal cavity and heart.

TABLE 7

GERMICIDAL ACTIVITY OF MOUTH WASH OF A 1:20,000 DILUTION OF ETHYLHYDROCUPREIN HYDROCHLORID IN 1:20 LIQUOR THYMOLIS ON PNEUMONIC SPUTUM CONTAINING VIRULENT TYPE I ORGANISMS

Exposure at 37 C.	Animal Tests; Days			
	1	2	3	4
1 minute	S	D*		
2 minutes	D*			
3 minutes	S	D*		
5 minutes	D			
10 minutes	S	S	S	S
Control (1 minute)	D*			
Control (10 minutes)	D*			

* Type I pneumococci in peritoneum and heart.

3 minutes with a 1:20,000 solution of ethylhydrocuprein hydrochlorid in 1:10 liquor thymolis or 1:10,000 solutions of quinin bisulphate or quinin hydrobromid followed by rinsing the mouth with sterile water and collection of sputum, showed a sufficient number of Type IV pneumococci in 1 c.c. of mouth secretion to kill mice within 24 hours with pneumococci in the blood of the heart. With a wash of 1:10,000 ethylhydrocuprein hydrochlorid followed by rinsing the mouth with sterile water and collection of sputum, 0.7 c.c. of sputum at times failed

to kill mice, whereas 0.7 c.c. of sputum collected before using the wash invariably killed within 24 hours with pneumococci in the blood of the heart. Larger doses of sputum collected after using the wash also killed, indicating that the wash had not destroyed all of the pneumococci, but probably had succeeded in reducing their numbers.

Bearing in mind the numerous difficulties in disinfecting the mouth of pneumococci even with powerful and more or less specific anti-pneumococcus agencies as ethylhydrocuprein and other quinin compounds, it is hardly to be expected that complete destruction of all pneumococci in the mouth and upper air passages generally can be accomplished by this means, but for use among physicians, nurses and members of a family in intimate contact with persons suffering with lobar pneumonia the systematic and daily use of washes prepared from 1:10,000 solutions of ethylhydrocuprein hydrochlorid or quinin bisulphate in 1:10 liquor thymolis, *may serve to destroy virulent pneumococci as they gain access to the mucous membrane of the mouth and upper part of the throat and prevent their proliferation in large numbers;* in this manner and among such groups of persons the systematic use of a mouth wash of this kind held in the mouth and gargled in the throat for at least a minute twice or three times each day may aid in the prophylaxis of lobar pneumonia. Ethylhydrocuprein hydrochlorid by reason of its superior pneumococcidal properties is to be preferred, but owing to the great scarcity of the drug at this time may be substituted by quinin bisulphate; solutions of either stronger than 1:10,000 are likely to prove objectionable to most persons. Liquor thymolis in itself appears to aid in the disinfecting process and is well borne in a 1:10 dilution serving also to disguise to a large extent the bitter taste of ethylhydrocuprein or other cinchonic.

CONCLUSIONS

Experiments in vitro have shown the high pneumococcidal activity of varying dilutions of ethylhydrocuprein hydrochlorid, quinin bisulphate and other cinchona compounds in liquor thymolis, for Type I and Type IV pneumococci in the sputum of convalescents from lobar pneumonia and the mouth secretions of normal persons.

As recent investigations in the epidemiology of lobar pneumonia have indicated that the disease is frequently acquired by transmission of specific virulent types of pneumococci from a recovered person who still harbors in the mouth secretions the organisms responsible for the

disease, or from a healthy carrier of pathogenic pneumococci, it is suggested that the systematic use by the patient and those in close contact with the disease of a mouth wash of 1:10,000 solution of ethylhydrocuprein hydrochlorid or quinin bisulphate in 1:10 liquor thymolis, may serve to destroy or inhibit the multiplication of the antisepticus, may serve to destroy or inhibit the multiplication of the disease producing pneumococci and thereby aid in the prophylaxis of lobar pneumonia.